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MASS SPECTRAL INVESTIGATIONS ON TOXINS

II. SIMULTANEOUS DETECTION AND QUANTIFICATION OF ULTRA-TRACE LEVELS OF SIMPLE TRICHOTHECENES IN ENVIRONMENTAL AND FERMENTATION SAMPLES BY GAS CHROMATOGRAPHIC/NEGATIVE ION CHEMICAL IONIZATION-MASS SPECTROMETRIC TECHNIQUES

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A fast, sensitive, and accurate gas chromatographic/mass spectrometric (GC/MS) method for simultaneously detecting and quantifying several simple trichothecene mycotoxins and related molecules has been developed. The method converts the polar trichothecenes into their volatile, stable heptafluorobutyrl (HFB) esters and follows the conversion with a GC/MS analysis of the derivatives. The derivatives were ionized under chemical ionization conditions, and the negative ions formed during the ionization were monitored. The samples were screened for trichothecene presence by the selected ion monitoring (SIM) mode, and the detected compounds were confirmed either by SIM or total ion monitoring. Two semisynthetic trichothecenes (deoxyverrucarol and 16-hydroxyverrucarol) were investigated and found adequate as internal standards for detecting and quantifying these toxic compounds. Femtograms and low picogram quantities (1-5 pg) of these compounds could be
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Detection limits

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detected and confirmed, respectively, by this procedure. A short cleanup procedure using silica-gel SEP-PAK cartridges was developed and used for the accurate analysis of real-world samples. Several spike and a few real-world samples were analyzed by this method with excellent sensitivity and precision. (keywords:)

PREFACE

The work described in this report was authorized under Project No. 1L161101A91A, In-House Laboratory Independent Research, Technical Area 3-A. This work was started in November 1982 and completed in January 1984.

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This report has been approved for release to the public.

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1. INTRODUCTION

Trichothecene mycotoxins, products of several genera of imperfect fungi, are toxic, chemically stable compounds with a characteristic tetracyclic 12,13-trichothec-9-ene ring structure.[1-6] Numerous trichothecenes have been isolated and well characterized. Based on their structural features, mycotoxins have been divided into two major groups. The two groups, depending upon their structures,[1] are designated as simple or macrocyclic trichothecenes. The simple trichothecenes possess hydroxyl, acetyl, and/or other ester groups. A keto group will be present at position 8 in some compounds (Table 1). The molecules of the other type are di-, or triesters of fusarium trichothecenes. These molecules are characterized by large ester bridges and called macrocyclic trichothecenes.[2]

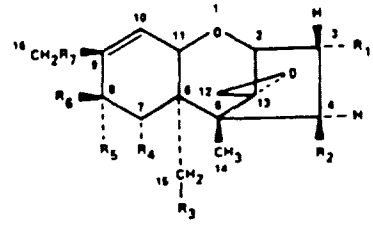
The health hazards associated with these fungal metabolites pose serious problems for animals and humans throughout the world.[2-14] Agricultural scientists have reported crop losses due to the damage arising from molds and animal losses due to farm animals consuming infested feed materials.[15-18] Monitoring farm and dairy products to detect these toxins and prevent economic losses has become very essential and routine in agricultural industries.[17-20] Intense research on the chemistry, chemical reactions, analysis, and toxicity of these compounds has resulted in several review articles and books being published.[5,13,21-26]

Recently, the alleged threat to humans in various parts of the world by induced trichothecenes (yellow rain) has been brought to the general public's attention.[27,28] Articles published in technical journals confirm the presence of some fusarium trichothecenes in yellow rain samples and samples of blood from attack victims.[29,30]

Thus, the threat to humans and animals due to these toxins, whether from natural or induced sources, has been clearly documented and demonstrated. Hence, to detect the trichothecenes and prevent natural and/or induced catastrophies, fast, specific, sensitive, accurate, and reliable methods for detecting and quantifying these toxins in various sample matrices need to be developed.

After careful review and evaluation of the available gas chromatographic/mass spectrometric (GC/MS) analytical methodologies, we pursued a different approach and developed a simple,

Table 1. Simple Trichothecenes and Related Compounds



COMPOUND ABBREVIATION	MOLECULAR WEIGHT	R_1	R_2	R_3	R_4	R_5	R_6	R_7
4-DEOXYVERRUCAROL (DOVE)	250	H	H	OH	H	H	H	H
VERRUCAROL (VER)	266	H	OH	OH	H	H	H	H
SCIRPENTRIOL (3o HOVER)	282	OH	OH	OH	H	H	H	H
8o-HYDROXYVERRUCAROL (8o HOVER)	282	H	OH	OH	H	OH	H	H
8β-HYDROXYVERRUCAROL (8β HOVER)	282	H	OH	OH	H	H	OH	H
16-HYDROXYVERRUCAROL (16 HOVER)	282	H	OH	OH	H	H	H	OH
9, 10-EPOXYVERRUCAROL (EPVER)	282	H	OH	OH	H	H	H	H
8-KETOVERRUCAROL (8 KEVER)	280	H	OH	OH	H	=O		H
DEOXYNIVALENOL (DON)	296	OH	H	OH	OH	=O		H
FUSARINON-X (FUSX)	354	OH	OAc	OH	OH	=O	H	H
MONOACETOXYSCIRPENOL (MAS)	324	OH	OH	H	H	H	H	H
DIACETOXYSCIRPENOL (DAS)	366	OH	OAc	OAc	H	H	H	H
T-2	424	OH	OH	OAc	H	$Me_2CHCH_2C(=O)O$	H	H
T-2	466	OH	OAc	OAc	H	$Me_2CHCH_2C(=O)-O$	H	H

reproducible, and sensitive method. This method uses high resolution gas chromatographic/negative ion chemical ionization-mass spectrometric (HRGC/NICI-MS) techniques for simultaneously detecting, confirming, and quantifying these fusarium trichothecenes and related molecules. The method converts samples containing mixtures of polar, simple trichothecenes and related compounds into their volatile heptafluorobutyryl (HFB) esters and analyzes them by the GC/NICI-MS techniques. The observed trichothecenes would be quantified using molecules structurally similar to naturally occurring compounds as internal standards. Ultra-trace levels [0.2-5.0 picogram (pg)] of simple trichothecenes were detected, confirmed, and quantified using this procedure.

We investigated two semisynthetic trichothecenes [deoxyverrucarol (DOVE) and 16-hydroxyverrucarol (HOVER)] and found them adequate for use as internal standards for detecting and quantifying simple trichothecenes in real-world samples. The developed procedures have been used successfully for the accurate, quantitative analyses of several matrix spiked (blind) samples and some environmental ones. In this report, we describe a simple, fast, reliable GC/NICI-MS procedure for routinely analyzing samples in a mass spectrometric (MS) laboratory with limited available instrumentation.

2. EXPERIMENTAL

All the GC/MS measurements were made using a Hewlett-Packard (HP) 5985-B MS. Fused silica capillary columns from J&W Associates (Ventura, CA) were used throughout the investigations. Ultra-pure solvents (Burdick & Jackson, Muskegon, MI), derivatizing agents (Regis Chemical Company, Chicago, IL), micro glassware (Supelco, Incorporated, Supelco Park, PA), and SEP-PAK cartridges (Waters Associates, Incorporated, Milford, MA) were used. Several of the fusarium trichothecene standards including DOVE and HOVER were generously provided by Professor Bruce Jarvis (University of Maryland, College Park, MD). Scirpentriol and fusarinon-X were obtained from Professor Chester Mirocha (University of Minnesota, Minneapolis, MN).

The trichothecene standard stock solutions were made at 2 °C in methanol in reacti-vials (Supelco, Incorporated) fitted with mininert valves. Diluted standard solutions were prepared using microsyringes. Standard solutions containing all trichothecenes in concentrations of 1 ng/µl and 10 ng/µl were frequently made and used immediately. Internal standards (10 ng/µl) (DOVE and HOVER) were also made frequently, and the same solutions were used for each series of experiments. Under appropriate conditions, a solvent wash or air column technique was followed to accurately transfer standards by microsyringes.

2.1 Derivatization Procedure.

Solutions [1-1000 nanogram (ng)] of standard mixture and internal standards (100 ng) were transferred into a 1.5-ml vial with

a Teflon-lined screw cap. The solvent evaporated under nitrogen, and the residue was treated with 0.5 ml of 10% acetonitrile/toluene and HFB imidazole (HFBI, 0.25-0.5 µl). The reaction mixture was shaken well once and kept in a heating block at 110 °C for 15 min. After cooling, the mixture was washed twice with 0.4 ml of 5% $NaHCO_3$ solution and 0.5 ml of distilled water. After each washing, the aqueous layer was removed using a disposable pipette. The organic layer containing the HFB esters was kept in the freezer at -4 °C until used. Samples were also derivatized under the same conditions using a slight excess of HFBI for an assured total derivatization.

2.2 Extraction and Cleanup of Spiked or Real Samples.

Samples were extracted with methanol (3 x 20 ml), and combined extracts were evaporated under nitrogen at 50 °C. The residue was dissolved using a minimum amount of 5% methanol/methylene chloride. The SEP-PAK cartridge was washed once with the same solvent, and the washings were discarded. The sample solution was passed through the cartridge, and the eluant was collected. The cartridge was washed twice more with 10 ml of the solvent. The combined eluants were evaporated in a 3.5-ml vial under nitrogen at 50 °C, and the residue was derivatized and stored as mentioned earlier in this report.

2.3 Analysis of HFB Esters.

Using the solvent (toluene - 1 µl) wash technique, 1 µl of the derivative mixture was injected into the HP gas chromatograph fitted with a grob injector in the splitless mode. A DB-5 fused silica (30 m, 0.25-mm i.d., 0.25 micron film thickness) capillary column, directly interfaced to the ionization source, was used throughout this investigation. An SE-54 fused silica capillary column with the same dimensions as those for the DB-5 capillary was also adequate when it was similarly used. The GC column heating was programmed from 150 °C (held for 1 min) to 300 °C (held for 10 min). The resolved esters were introduced into the source and subjected to chemical ionization (CI) at 100 °C using methane (0.5-1.0 torr) as the CI reagent gas. The MS was controlled and operated by the data system during monitoring of either total or selected ions. The ion currents or the area of the peaks due to specific ions were measured and used.

3. RESULTS AND DISCUSSION

Some of the trichothecenes listed in Table 1 are products of the cultures of fusarium fungi. The others are either hydrolytic products of macrocyclic trichothecenes or semisynthetic compounds. Scirpentriol (3α-HOVER), deoxynivalenol (DON), fusarinon-X (FUSX), monoacetoxyscirpenol (MAS) and diacetoxyscirpenol (DAS), HT-2, and T-2 are naturally occurring toxins.[1] Verrucarol and the substituted verrucarols are the hydrolytic products of the corresponding macrocyclic trichothecenes.[2] DOVE is a semisynthetic product derived from either

verrucarol or DAS.[31] HOVER is one of the hydrolytic products of 16-hydroxyverrucarin A, which, in turn, is obtained by the allylic oxidation of naturally occurring verrucarin A using selenium dioxide[32] or microbial transformation of verrucarin A.[33] We investigated these two synthetically obtained, simple trichothecenes for use as potential internal standards for identifying and quantifying simple trichothecenes and related molecules.

Optimum conditions for converting fusarium trichothecenes into their corresponding HFB esters were determined by varying the reaction temperature and time, the concentration of acetonitrile, and the quantity of the derivatizing agent (HFBI). The number of derivatives formed was determined by the negative ion chemical ionization (NICI) mode during the GC/MS analysis which monitored single characteristic ions for each molecule. The relative intensities of these ions with respect to m/z 426 (DOVE) were used as the criteria to determine the extent of the derivatization. The HFB esters were effectively formed by heating the trichothecenes with HFBI in 10% acetonitrile/toluene at 100 °C for 15 min. The clear solution containing the derivatives, obtained after repeatedly washing the reaction mixture with 5% aqueous $NaHCO_3$ solutions and distilled water, was adequate for the GC/MS analysis. The mixture containing the HFB esters was injected into the gas chromatograph, and the chromatographically separated derivatives were analyzed by mass spectrometry. Along with the chromatographic conditions, the reconstructed total ion chromatograms are indicated in Figures 1 and 2. The electron impacts of the chromatograms [(EI) 200 °C], positive ion chemical ionization (PICI), and NICI (methane) (100 °C, 0.5-1.0 torr) spectra were recorded and characterized. To monitor the higher mass ions with increased sensitivity, only the mass spectra of the esters in the mass range of m/z 200-950 were recorded. Sixteen of the ions with higher relative abundances were selected for tabulating. In this selection, preference was given to higher mass ions whenever the number of higher intensity ions exceeded 16. These partial mass spectra are indicated in Tables 2-4.

As expected, the partial EI spectra provide only limited structural information on the HFB derivatives. The verrucarols seem to form ions by removing one or more heptafluorobutyric acid (HFBH) molecules and other neutral molecules such as $CH_2=C=O$. Similar fragmentations were also noted in T-2 and HT-2 toxins.

In the PICI spectra of FUSX and DON, the most abundant ion seemed to come from the protonated HFBH ions formed by hydrogen rearrangement in the protonated molecules. This observation agrees with the one Munson and Field[34] made of esters. In verrucarol and DOVE, the most abundant ions were formed by the removal of the HF molecule; in MAS and DAS, by the removal of CH_3COOH; and in T-2 and HT-2, by the removal of isovaleric acid and CH_3COOH. In all cases, the $(M+C_2H_5)^+$ and $(M+C_3H_5)^+$ adducts were noted along with ions formed by the removal of HFBH(s), CO, $CH_2=CH_2$, CH_3COOH, and isovaleric acid from the quasimolecular ions.

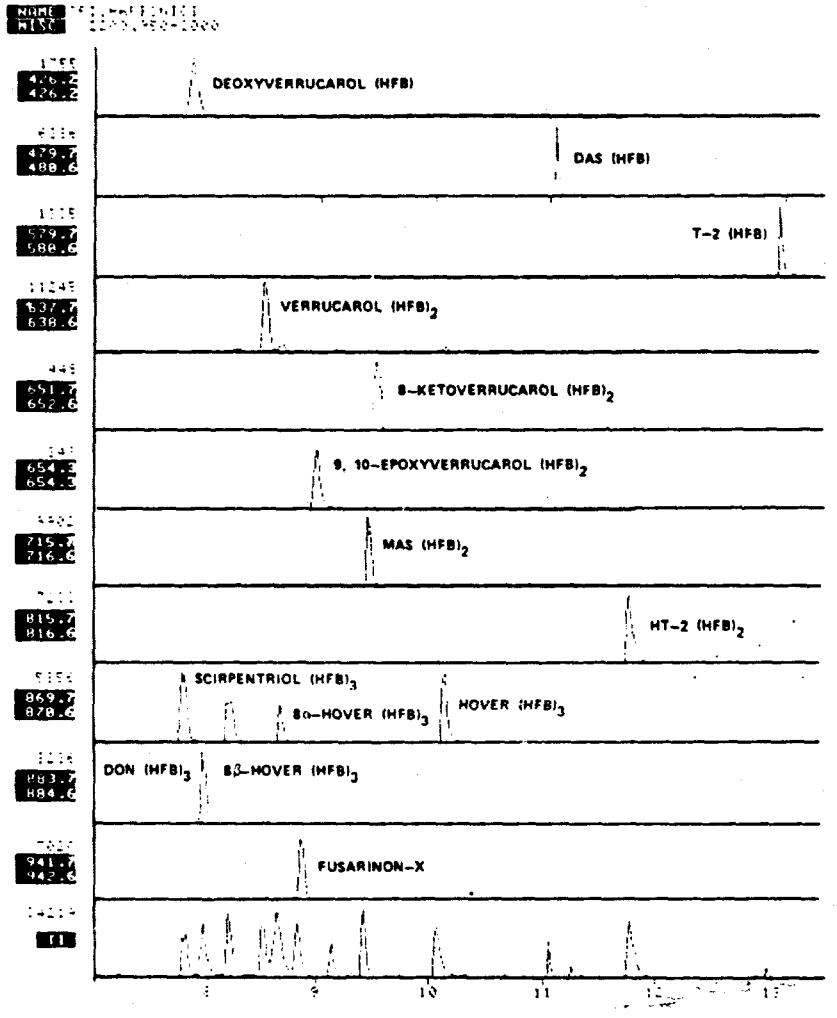


Figure 1. RIC of HFB Derivatives of Simple Trichothecenes. [DB-5 Fused Silica Capillary Column (0.25 mm x 30 m, 0.25μ). Splitless injection; column temperature - held at 150 °C for 1 min, heated at 10 degrees/min for 10 min, heated to 300 °C at 25 degrees/min, held for 10 min]

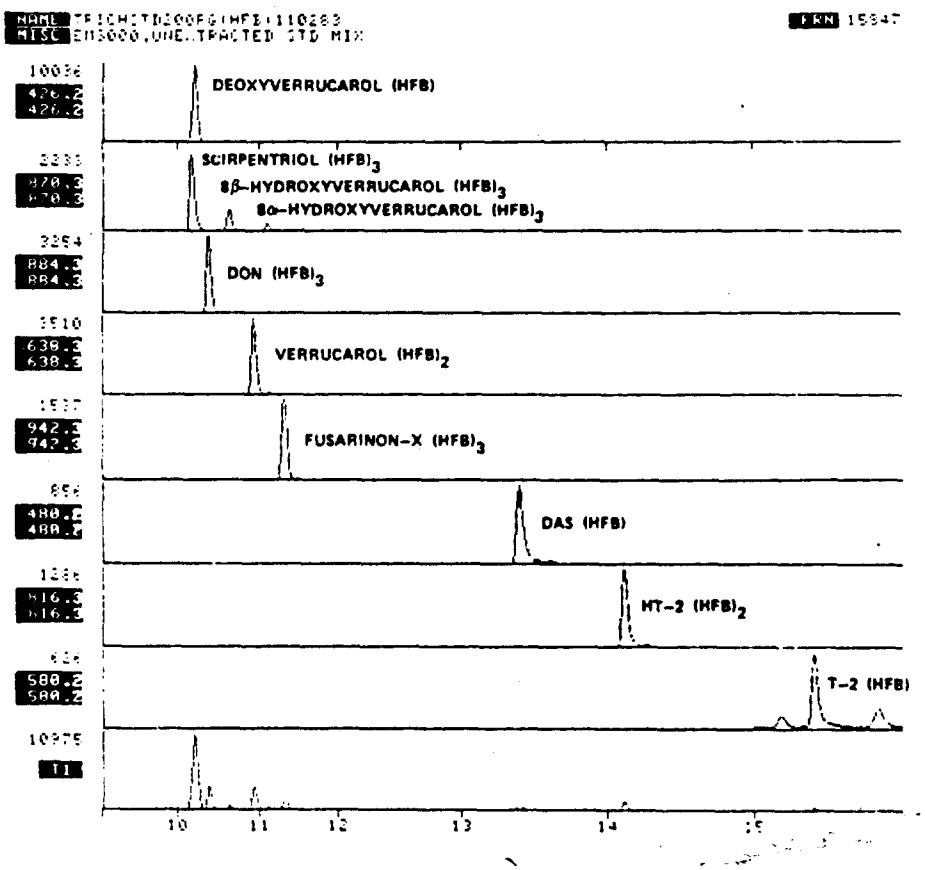


Figure 2. RIC of HFB Esters of Fusarium Trichothecenes. [SE-54 Fused Silica Capillary Column (30 m x 0.25 mm i.d., 0.25μ). Splitless injection; column temperature - held at 150 °C for 2 min, heated at 10 degrees/min for 10 min, heated to 300 °C at 25 degrees/min, held for 10 min]

Table 2. Electron Impact Mass Spectra

Compound	Molecular Weight	Base Peak (m/z)	Significant Ions (Relative Abundance) (m/z)
4-Deoxyverrucarol (HFB)	446	204	446(31.3), 432(19.2), 431(88.5), 417(3.2), 403(4.8), 390(2.7), 347(6.2), 335(4.1), 318(3.0), 305(2.0), 281(6.5), 232(60.6), 217(15.0), 205(19.9), 201(14.7)
Verrucarol $(HFB)_2$	658	318	658(19.9), 643(61.5), 445(56.1), 444(77.2), 429(14.3), 416(37.9), 403(13.0), 361(21.1), 345(20.5), 331(53.4), 321(32.5), 305(60.0), 292(42.7), 281(36.9), 231(44.5), 215(41.8)
Scripentriol $(HFB)_3$	87	318	885(10.8), 656(62.4), 628(31.5), 443(14.7), 415(23.4), 399(12.9), 396(16.5), 385(11.1), 368(15.7), 359(14.4), 343(11.8), 337(17.5), 335(26.3), 331(17.5), 304(32.0), 253(24.3)
8 α-Hydroxyverrucarol $(HFB)_3$	870	317	657(10.5), 656(9.1), 443(12.3), 429(8.9), 387(2.6), 361(2.0), 345(3.0), 331(9.7), 318(16.2), 309(10.1), 292(13.3), 281(5.2), 267(2.6), 229(8.7), 215(11.4), 201(11.8)

Table 2. Electron Impact Mass Spectra (Continued)

Compound	Molecular Weight	Base Peak (m/z)	Significant Ions (Relative Abundance) (m/z)
8 β-Hydroxyverrucarol $(HFB)_3$	870	317	657(99.0), 444(18.9), 443(86.1), 429(76.7), 416(17.1), 387(17.1), 375(12.6), 361(14.2), 347(25.8), 331(25.1), 321(20.8), 305(37.0), 293(23.1), 280(32.4), 229(58.4), 215(70.8)
16-Hydroxyverrucarol $(HFB)_3$	870	317	658(13.8), 657(66.2), 656(94.2), 443(11.8), 429(10.4), 415(7.4), 387(10.1), 345(17.8), 331(27.7), 321(16.6), 305(35.5), 292(15.6), 281(22.3), 229(39.8), 215(78.3), 205(1.9)
Deoxynivalenol $(HFB)_3$	884	294	884(23.8), 670(5.0), 651(5.0), 643(9.6), 615(7.3), 573(11.9), 457(8.4), 377(10.0), 359(67.4), 347(14.2), 333(72.0), 331(80.8), 305(54.8), 253(31.8), 213(19.9), 207(21.5)
Fusarinone-X $(HFB)_3$	942	294	942(4.3), 701(3.4), 670(7.9), 669(33.0), 627(13.4), 589(10.4), 413(14.3), 385(12.6), 375(23.7), 357(26.7), 347(21.1),333(40.3), 321(66.1), 320(26.9), 267(17.5), 253(16.4)
Monacetoxyscirpenol $(HFB)_2$	716	656	673(3.1), 657(26.4), 641(13.7), 628(15.3), 443(34.3), 429(43.9), 415(37.0), 396(14.7), 385(17.0), 368(19.8), 304(33.5), 291(11.3), 267(14.5), 239(10.0), 229(42.8), 201(51.2)

Table 2. Electron Impact Mass Spectra (Continued)

Compound	Molecular Weight	Base Peak (m/z)	Significant Ions (Relative Abundance) (m/z)
Diacetoxyscirpenol (HFB)	562	502	503(22.4), 474(11.5), 459(12.4), 430(10.2), 429(43.2), 415(11.8), 396(10.5), 368(9.6), 321(20.9), 317(13.0), 304(13.0), 289(11.5), 216(13.8), 229(57.0), 217(41.3), 205(58.3)
HT-2$(HFB)_2$	816	205	672(1.3), 655(3.9), 654(2.0), 441(2.1), 427(2.8), 371(1.3), 320(2.1), 304(1.7), 281(2.0), 267(6.0), 264(3.8), 253(2.7), 245(2.3), 227(7.3), 213(9.9), 201(9.6)
T-2(HFB)	662	205	578(1.3), 560(1.0), 518(3.5), 501(8.2), 427(6.9), 397(2.0), 381(5.2), 320(11.0), 267(10.1), 264(10.9), 245(14.9), 227(31.2), 215(15.9), 213(24.0), 207(40.6), 201(29.0)

Table 3. Positive Ion Chemical Ionization Mass Spectra

Compound	Molecular Weight	Base Peak (m/z)	Significant Ions (Relative Abundance) (m/z)
4-Deoxyverrucarol (HFB)	445	233	487(3.3), 475(3.3), 448(15.0), 447(98.8), 446(13.1), 429(13.0), 417(4.6), 411(1.6), 401(2.7), 399(2.4), 277(1.7), 249(17.1), 234(17.7), 219(1.4), 215(27.0), 203(7.0)
Verrucarol $(HFB)_2$	658	445	659(14.0), 641(2.9), 501(1.0), 489(1.4), 461(12.8), 446(22.7), 429(2.0), 427(6.0), 417(8.5), 323(2.9), 301(1.8), 293(1.5), 247(19.0), 233(6.3), 231(28.7), 215(29.2)
Scripentriol $(HFB)_3$	870	215	911(1.3), 899(1.2), 871(22.7), 853(7.8), 841(3.4), 673(4.9), 657(92.2), 639(6.9), 627(2.7), 535(13.7), 461(5.3), 443(25.2), 425(4.7), 415(4.0), 337(6.4), 233(14.0)
8 α-Hydroxyverrucarol $(HFB)_2$	870	215	871(2.5), 853(1.0), 657(50.7), 461(3.4), 445(13.2), 443(9.7), 425(2.6), 339(1.4), 318(3.2), 277(1.5), 261(2.1), 249(3.9), 243(7.0), 233(12.1), 229(19.6), 207(3.0)

Table 3. Positive Ion Chemical Ionization Mass Spectra

Compound	Molecular Weight	Base Peak (m/z)	Significant Ions (Relative Abundance) (m/z)
8 β-Hydroxyverrucarol $(HFB)_3$	870	215	899(1.0), 871(7.1), 673(2.5), 659(7.0), 658(21.1), 675(76.5), 639(1.7), 619(1.3), 461(2.7), 445(8.6), 443(16.7), 339(3.2), 247(6.7), 243(8.0), 233(1.0), 229(14.6)
16-Hydroxyverrucarol $(HFB)_3$	870	215	871(5.0), 657(60.1), 639(3.0), 627(1.4), 461(2.4), 459(3.2), 445(10.3), 443(17.7), 427(2.3), 425(3.0), 415(2.0), 323(2.0), 319(1.9), 247(4.0), 243(8.0), 233(15.1)
Deoxynivalenol $(HFB)_3$	884	215	925(2.0), 913(2.2), 886(12.2), 885(37.2), 672(4.9), 671(7.5), 643(1.6), 499(2.3), 487(2.9), 459(24.7), 334(1.1), 291(1.4), 263(1.3) 245(5.2), 233(11.5), 229(6.1)
Fursarinon-X $(HFB)_3$	942	215	943(38.7), 883(1.5), 730(5.1), 661(1.1), 745(1.7), 517(18.9), 459(1.5), 445(1.6), 321(1.1), 303(1.4), 273(1.8), 261(2.4), 247(2.8), 243(4.9), 233(8.5), 229(12.1)
Monoacetoxyscirpenol $(HFB)_2$	716	657	757(7.6), 745(6.8), 717(81.0), 699(5.4), 685(2.8), 568(26.9), 639(10.3), 619(2.6), 563(1.7), 535(2.5), 503(15.5), 485(2.8), 465(2.4), 443(8.2), 425(4.3), 289(8.2)

Table 4. Negative Ion Chemical Ionization Mass Spectra

Compound	Molecular Weight	Base Peak (m/z)	Significant Ions (Relative Abundance) (m/z)
4-Deoxyverrucarol (HFB)	446	426	446(0.5), 428(3.5), 427(20.5), 408(1.0), 213(1.6)
Verrucarol$(HFB)_2$	658	638	660(1.9), 659(9.9), 658(35.3), 640(5.8), 639(25.3), 621(1.1), 620(3.2), 618(1.9), 441(2.3), 440(9.3), 213(19.1)
Scirpenetriol$(HFB)_3$	870	870	872(6.6), 871(28.2), 852(4.9), 851(20.6), 850(51.4), 832(7.9), 814(1.2), 682(1.4), 674(5.1), 654(1.1), 638(2.2), 637(5.6), 618(3.1), 440(3.6), 233(2.3), 213(53.8)
8 α-Hydroxyverrucarol$(HFB)_3$	870	870	871(31.9), 851(20.2), 832(3.3), 670(2.6), 658(18.5), 654(8.0), 638(42.6), 620(3.2), 563(1.4), 544(14.2), 440(1.4), 434(2.1), 404(3.3), 375(2.0), 216(57.3), 213(57.3)
8 β-Hydroxyverrucarol$(HFB)_3$	870	870	871(21.3), 851(15.7), 850(59.8), 658(17.5), 656(12.1) 652(3.5), 638(42.8), 636(24.9), 618(5.4), 471(2.6), 463(2.4), 442(13.1), 440(12.3), 422(22.8). 402(15.4), 213(97.1)

Table 4. Negative Ion Chemical Ionization Mass Spectra (Continued)

Compound	Molecular Weight	Base Peak (m/z)	Significant Ions (Relative Abundance) (m/z)
16-Hydroxyverrucarol$(HFB)_3$	870	870	871(29.5), 851(14.9), 850(49.3), 830(3.7), 810(3.5), 696(1.0), 674(2.9), 659(2.4), 658(9.2), 652(2.2), 638(18.3), 602(1.6), 478(2.9), 440(2.6), 404(1.2), 213(62.8)
9,10-Epoxverrucarol$(HFB)_2$	870	654	675(6.4), 684(17.8), 656(4.8), 655(26.3), 636(2.6), 635(1.4), 634(4.5), 476(1.0), 440(2.4), 436(1.2), 216(1.0), 214(1.7), 213(1.0)
8-Ketoverrucarol$(HFB)_2$	672	652	672(11.5), 654(8.1), 653(28.7), 633(7.0), 632(13.8), 613(3.9), 438(20.2), 420(4.2), 213(25.0)
DDD$(HFB)_2$	774	213	775(3.2), 774(9.5), 754(1.6), 585(1.4), 581(5.0), 580(13.7), 577(1.0), 560(3.8), 540(2.7), 520(12.0), 425(1.7), 362(1.3), 244(1.3), 233(1.6), 216(25.7), 204(2.2)

Table 4. Negative Ion Chemical Ionization Mass Spectra (Continued)

Compound	Molecular Weight	Base Peak (m/z)	Significant Ions (Relative Abundance) (m/z)
Deoxynivalenol(HFB)$_3$	844	844	885(27.8), 864(2.4), 844 (1.3), 688 (1.2), 672(9.6), 670(4.9), 651(3.6), 632(2.5), 630(1.3), 460(2.8), 459(12.6), 458(57.5), 438(2.6), 420(1.7), 262(1.4), 213(48.5)
Fusarinon-X (HFB)$_3$	942	942	943(31.1), 922(2.1), 902(1.3), 870(3.9), 816(11.9), 730(12.0), 728(4.7), 716(5.4), 699(1.6), 658(2.5), 638(5.9), 517(13.5), 516(53.2), 458(26.1), 320(2.2), 213(39.0)
Monoacetoxyscirpenol(HFB)$_2$	716	716	718(5.6), 717(26.1), 698(2.1), 697(3.2), 696(7.5), 679(1.6), 678(6.7), 660(1.0), 518(1.0), 483(1.0), 233(5.4), 216(2.9), 214(2.7), 213(43.0)
Diacetoxyscirpenol(HFB)	562	480	562(1.8), 544(2.2), 543(11.9), 542(41.4), 524(1.9), 523(2.0), 522(6.9), 495(1.1), 484(1.7), 483(5.1), 482(5.6), 481(23.1), 436(3.5), 364(1.0), 237(7.5), 213(6.4)

Table 4. Negative Ion Chemical Ionization Mass Spectra (Continued)

Compound	Molecular Weight	Base Peak (m/z)	Significant Ions (Relative Abundance) (m/z)
HT-2(HFB)$_2$	816	816	802(1.2), 796(9.6), 778(3.1), 733(1.4), 716(18.3), 696(3.9), 678(5.2), 662(5.2), 642(24.6), 622(3.5), 600(1.7), 582(6.1), 580(78.3), 542(2.6), 536(2.4), 213(53.2)
T-2(HFB)	662	580	662(4.8), 643(6.0), 642(23.7), 622(4.4), 581(31.2), 562(1.8), 542(4.7), 540(2.1), 536(3.3), 522(1.4), 502(1.0), 498(4.8), 480(9.1), 436(1.9), 237(1.9), 213(7.8)

The NICI spectra of these molecules indicate that in most instances, the most abundant ions were either M^- or $(M-HF)^-$ ions with the exception of DAS and T-2 when $(M-CH_3COOH)^-$ ions were the most predominant. The other commonly observed ions were formed by the removal of HF(s), HFBH(s), and H_2O. As expected, there are fewer ions, and the higher mass ions are predominant in NICI spectra of all the derivatives. Hence, we pursued our investigation and developed a method for simultaneously analyzing and quantifying several ultra-trace levels of trichothecenes by NICI.

A mixture containing 100 ng of the trichothecenes and 100 ng of the internal standards was derivatized and analyzed periodically over 18 days to determine the stability of the derivatives under the planned experimental conditions. The derivatives were analyzed by the selected ion monitoring (SIM) mode, monitoring a single ion for each molecule. The MS was tuned specifically to enhance the higher mass ions. No other distinct quality control steps were taken during the tuning. The instrumental operational conditions adapted during the analysis are listed in Table 5. The classification of groups was based on the elution times of the molecules and the intensities of their corresponding ions. More time was given to monitor less intense ions. The derivatives were stored in the freezer (-4 °C) at all times except during analysis. The 1-µl solution containing the HFB esters of analytes and internal standards was analyzed by the SIM mode at least four consecutive times, and the intensities of all the ions were measured. The average of the relative intensities of the individual ions with respect to DOVE (m/z 426) and HOVER (m/z 870) were calculated, and these values are shown in Table 6. Under the chromatographic conditions used for this investigation, the adsorption of the derivatives on the DB-5 fused silica capillary GC column was nil. A similar phenomenon was also noted while using SE-54 fused silica capillary columns. Hence, the difference between the observed relative intensities of the first and second measurements was negligible while using either column.

Despite the changes in relative intensities due to variances in their stabilities over a period of time, the measured relative intensities of various HFB esters and the observed standard deviations in the measurements indicate that these values could be obtained with excellent precision. The stabilities of the derivatives with the exception of scirpentriol are comparable with those of the DOVE(HFB) over 4 days. This internal standard seems adequate over 18 days for all trichothecenes except 3α-HOVER, 8ß-Hydroxyverrucarol, and FUSX. The relative intensities of these trichothecenes with respect to HOVER$(HFB)_3$ could also be measured with good accuracy within 48 hr. Beyond this period, the introduced error in these measurements is at least 15% or more for 3α-HOVER, 8ß-HOVER, DON, FUSX, and DAS. The variation in these values seemed to be higher when HOVER(HFB) was used as the internal standard. At this point, it is not clear whether the variation is due to the instability of the derivatives or to the

Table 5. Instrumental Conditions for Screening the Simple Trichothecenes

Group	Start Time (min)	Run Time (min)	Compounds Monitored	Mass (Dwell Time) m/z (msec)
1	7.0	1.6	Dove	462.2 (50)
			Scirpentriol	870.3 (50)
			8β-HOVER	870.3 (50)
			DON	884.3 (50)
2	8.6	2.2	Verrucarol	638.3 (50)
			8α-HOVER	870.3 (50)
			HOVER	870.3 (50)
			FUSX	942.3 (50)
3	10.8	0.7	DAS	480.2 (150)
4	11.5	1.3	HT-2	816.3 (150)
5	12.8	1.0	T-2	580.2 (150)

Table 6. Stability Data of HFB Esters of Trichothecenes

COMPOUND	RELATIVE INTENSITIES* DAY 0	DAY 1	DAY 3	DAY 4	DAY 7	DAY 10	DAY 18
1. DOVE.HFB	0.86±0.06	0.75±0.13	0.75±0.08	0.75±0.10	0.91±0.90	0.46±0.00	0.53±0.05
2. VER.$(HFB)_2$	1.67±0.14	0.70±0.10	1.38±0.12	1.60±0.12	1.43±0.04	1.70±0.00	2.40±0.13
	(1.43±0.12)	(1.28±0.07)	(1.17±0.07)	(1.49±0.10)	(1.30±0.03)	(0.78±0.00)	(1.26±0.05)
3. 3α HOVER$(HFB)_3$	1.80±0.09	1.88±0.11	1.22±0.13	1.30±0.12	0.71±0.07	0.92±0.01	0.85±0.05
	(1.54±0.08)	(1.41±0.09)	(0.87±0.08)	(0.87±0.10)	(0.64±0.06)	(o.42±0.01)	(0.42±0.02)
4. 8α HOVER$(HFB)_3$	0.19±0.01	0.20±0.03	0.16±0.12	0.19±0.04	0.13±0.01	0.24±0.03	0.26±0.03
	(0.16±0.06)	(0.15±0.02)	(0.12±0.02)	(0.13±0.02)	(0.12±0.01)	(0.12±0.03)	(0.14±0.12)
5. 8β HOVER$(HFB)_3$	1.05±0.07	1.14±0.15	0.90±0.12	1.06±0.10	0.71±0.04	1.27±0.01	1.64±0.23
	(0.90±0.06)	(0.86±0.12)	(0.67±0.05)	(0.72±0.08)	(0.64±0.04)	(0.59±0.01)	(0.86±0.06)
6. HOVER$(HFB)_3$	1.17±0.07	1.33±0.17	1.35±0.14	1.39±0.17	1.10±0.10	1.98±0.27	1.91±0.17
9. DON$(HFB)_3$	3.90±0.80	4.30±0.48	4.03±0.56	3.96±0.64	2.22±0.13	3.59±0.01	3.59±0.01
	(3.34±0.09)	(3.16±0.36)	(2.91±0.19)	(2.85±0.20)	(2.01±0.12)	(1.65±0.00)	(1.68±0.05)
10. FUSX$(HFB)_3$	1.13±0.06	1.38±0.19	1.24±0.25	1.21±0.19	0.44±0.04	0.60±0.01	0.27±0.16
	(0.97±0.05)	(1.04±0.14)	(0.85±0.12)	(0.83±0.07)	(0.40±0.03)	(0.28±0.00)	(0.14±0.01)

Table 6. Stability Data of HFB Esters of Trichothecenes (Continued)

COMPOUND	RELATIVE INTENSITIES* DAY 0	DAY 1	DAY 3	DAY 4	DAY 7	DAY 10	DAY 18
11. MAS(HFB)$_2$	0.45±0.02	0.47±0.05	0.39±0.04	0.40±0.05	0.33±0.03	0.37±0.00	0.40±0.05
	(0.39±0.02)	(0.35±0.04)	(0.29±0.04)	(0.29±0.03)	(0.30±0.02)	(0.17±0.00)	(0.21±0.01)
12. DAS(HFB)	0.68±0.04	0.77±0.06	0.86±0.08	0.91±0.10	0.90±0.03	0.90±0.16	0.80±0.05
	(0.59±0.03)	(0.58±0.04)	(0.65±0.10)	(0.66±0.08)	(0.81±0.03)	(0.44±0.03)	(0.42±0.01)
13. HT-2(HFB)$_2$	1.04±0.08	1.51±0.19	1.46±0.24	1.39±0.14	1.04±0.08	1.69±0.40	1.30±0.18
	(0.89±0.07)	(1.13±0.15)	(1.08±0.08)	(1.08±0.13)	(0.82±0.07)	(0.82±0.12)	(0.67±0.06)
14. T-2(HFB)	0.40±0.06	0.54±0.07	0.58±0.06	0.53±0.08	0.50±0.06	0.91±0.00	0.57±0.06
	(0.35±0.05)	(0.41±0.05)	(0.43±0.04)	(0.38±0.04)	(0.45±0.05)	(0.45±0.01)	(0.29±0.01)

* Values with respect to DOVE(HFB)$_3$ and HOVER(HFB)$_3$. The latter values are listed in parenthesis.

adsorption of the HOVER. For an extended period, DOVE(HFB) proved to be a better internal standard over HOVER(HFB)$_3$. Readers should note that both internal standards seem adequate for all molecules studied for at least 48 hr after the derivatization. Thus, DOVE and HOVER (the two semisynthetic, structurally similar molecules with different polarities) could be used as internal standards for analyzing simple trichothecenes with varied polarities. In both cases, despite the error due to the uncontrolled MS tuning conditions, the relative intensities of most molecules over 2 weeks were measured with errors not exceeding 50%. This measurement is more than adequate for measurements of analytes present in low nanogram quantities. All the above results are clearly indicated in Table 6.

After establishing the optimum conditions for the synthesis, stability, and analysis of these derivatives, we conducted experiments to establish the minimum detectable and confirmable limits for each analyte. One characteristic ion for each derivative was monitored under the conditions listed in Table 5 for routinely monitoring samples to detect trichothecene presence. However, six specific ions were monitored for each compound to confirm trichothecene presence. During these SIM measurements, less intense ions were monitored with more dwell time to enhance their sensitivity. Along with their detectable and confirmable limits, the specific ions and their dwell times used to confirm trichothecene presence are listed in Table 7.

The following experiments were conducted to establish the concentration range in which the intensities of the observed ion currents vary linearly with the amount of the analytes. Varied amounts of trichothecene mixtures (2.5-250 ng) along with DOVE (50 ng) and HOVER (50 ng) were converted into their HFB esters. The resulting derivative mixtures contained 5-500 pg/µl of the trichothecene esters and 100 pg/µl of the internal standards. A 1-µl aliquot of the products was analyzed immediately by GC/NICI-MS under the conditions specified in Table 5. The ion currents of all the ions in various mixtures were measured. The ions' relative intensities with respect to both internal standards were calculated and plotted versus the ions' relative amounts. The constants obtained from the linear regression analysis of the data are shown in Table 8. In all instances, linearity existed between the relative intensities of the ions formed and the relative amounts of the esters analyzed. When the amount of analyte analyzed exceeded the 500-pg level, especially for DON, VER, and HT-2, the relative intensities of the ions detected did not follow the linear relationships with their relative amounts. When the values for 5-10 pg of concentration were removed from the linear regression analysis, better values for correlation coefficients and intercepts were obtained. Thus, the linear concentration range for all these derivatives has been established as 20-500 pg, and both internal standards seem to be adequate for the quantification. In addition, standard calibration curves were obtained with 100-500 pg/µl of DOVE and

Table 7. Minimum Detectable and Confirmable Limits for the HFB Esters

COMPOUND	Ions for Confirmation m/z (dwell time in millisecond)	Detection* (pg)	Confirmation (pg)
DOVE(HFB)	447.2(300), 446.2(300), 427.2(50), 426.2(50) 213.2(5)	1.0	2
VER(HFB)$_2$	559.3(50), 658.3(50), 639.3(50), 638.5(5), 440.2(150), 213.2(50)	0.25	1
SCIR(HFB)$_3$	871.3(50), 870.3(50), 851.3(50), 850.3(50) 832.3(150), 657.3(150), 637.3(150)	0.2	1
8R-HOVER(HFB)$_3$	871.3(50), 870.3(50), 850.3(50), 658.3(150) 638.5(50)	0.6	2
8 HOVER(HFB)$_3$	871.3(50), 870.3(50), 851.3(50), 850.3(50), 658.3(150), 638(50)	0.4	1
16 HOVER(HFB)$_3$	871.3(50), 870.3(50), 871.3(50), 850.3(50), 658(150), 638(50)	0.2	1
DON(HFB)$_3$	885.3(50), 884.3(50), 864.3(150), 651.3(150), 672.3(150), 458.2(150)	0.1	1
FUSX(HFB)$_3$	943.3(50), 942.3(50), 922.3(150), 902.3(150), 870.3(150), 728.3(150)	0.2	2

Table 7. Minimum Detectable and Confirmable Limits for the HFB Esters (Continued)

COMPOUND	Ions for Confirmation m/z (dwell time in millisecond)	Detection* (pg)	Confirmation (pg)
MAS(HFB)$_2$	717.3(50), 716.3(50), 697.3(150), 696.3(150), 678.3(150), 603.3(150)	0.5	2
DAS(HFB)	562.2(200), 543.2(150), 542.2(150), 522.2(150), 481.2(50), 480.2(50)	2.0	5
HT-2(HFB)$_2$	817.3(50), 816.3(50), 797.3(200), 796.8(200), 778.3(200), 716.3(100)	0.5	2
T-2(HFB)	662.3(200), 642.3(200), 622.3(200), 581.2(50), 580.2(50), 536.2(200)	2.0	5

*Conditions as listed in Table 5.

Table 8. Linear Regression Analysis Data of the Derivatives

COMPOUND (HFB ESTERS)	RT (min)	RRT 1	RRT 2	ANALYZED AMOUNT (pg)	CORRELATION COEFFICIENT 1	CORRELATION COEFFICIENT 2	SLOPE 1	SLOPE 2	INTERCEPT 1	INTERCEPT 2	RESPONSE FACTOR 1	RESPONSE FACTOR 2
Scirpenetriol	8.2	0.988	0.781	5-500	0.9981	0.9980	2.665	1.573	-0.244	-0.204	2.23±0.38	1.31±0.23
DOVE	8.3	1.000	0.790	"								
Deoxynivalenol	8.4	1.012	0.800	"	0.9965	0.9969	4.469	2.668	0.040	-0.068	4.15±0.42	2.21±0.31
8 β-Hydroxyverrucarol	8.6	1.036	0.819	"	0.9989	0.9909	1.594	1.179	-0.175	-0.383	1.34±0.23	0.74±0.17
Verrucarol	9.0	1.084	0.857	"	0.9993	0.9987	2.329	1.378	-0.145	-0.144	2.11±0.19	1.13±0.17
8 -Hydroxyverrucarol	9.1	1.096	0.867	"	0.9987	0.9991	0.359	0.213	-0.126	-0.085	0.25±0.08	0.15±0.04
Fusarinon-X	9.3	1.120	0.886	"	0.9989	0.9990	1.343	0.800	-0.053	-0.053	1.24±0.14	0.66±0.13
Monoacetoxyscirpenol(MAS)	9.9	1.193	0.942	"	0.9976	0.9972	0.780	0.468	-0.053	-0.030	0.77±0.08	0.42±0.37
16-Hydroxyverrucarol	10.5	1.264	1.000	"	0.9976	-	2.455	-	-0.305	-	1.87±0.20	-
Diacetoxyscirpenol	11.5	1.386	1.095	"	0.9992	0.9976	0.849	0.499	-0.042	-0.054	0.74±0.08	0.41±0.06
HT-2	12.1	1.458	1.152	"	0.9975	0.9966	2.302	1.360	-0.195	-0.168	2.19±0.16	1.13±0.14
T-2	13.4	1.614	1.276	"	0.9984	0.9985	0.632	0.377	-0.058	-0.054	0.53±0.07	0.30±0.06
	1-DOVE (IS)			2-HOVER(IS)								

100-250 pg/µl of HOVER. Varying the amount of internal standards had no adverse effect in the observed linearity. Most of the following investigations were conducted with internal standards concentrations of 100 pg/µl to minimize consumption of the standard materials.

The response factors of all these derivatives were calculated with respect to DOVE(HFB) and HOVER(HFB)$_3$; the values are listed in Table 8. Each reported response factor value resulted from 35 to 40 measurements of 10 solutions of different concentrations. The consistency in the response factor values measured and the precision of the measurements indicated by the low standard deviation values clearly establish the adequacy of the chosen internal standards and the stability of the synthesized derivatives.

Prior to developing the extraction and cleanup procedures for these fusarium trichothecenes, their stability after prolonged exposure to ambient conditions was checked. Solutions containing 25-100 ng of mixtures of trichothecene standards were exposed at room temperature in different 250-ml Erlenmeyer flasks for 7 days. The flasks were rinsed with hot methanol (3 x 10 ml), and the combined washings were transferred step-wise into a 2.5-ml vial and evaporated under nitrogen at 50 °C. The residues were treated with internal standards, derivatized, and analyzed. The amounts of individual trichothecenes were calculated by extrapolating their observed relative intensities into their corresponding calibration curves. The average of the six different observed recovery percentages for an individual trichothecene in a non-matrix situation are listed in Table 9. The recoveries, except for VER and MAS and the high standard deviations for DON and FUSX, were reasonably good.

Six residues containing the same amounts indicated in the previous experiments were dissolved in 5% methanol/methylene chloride (10-20 ml) and passed through a silica-gel SEP-PAK cartridge previously washed with the same solvent. The cartridge was washed again with the solvent (2 x 10 ml), and the combined washings were evaporated in a 2.5-ml vial. The residues with the internal standards were derivatized and analyzed, and the recovery percentages of individual compounds after the extraction and cleanup procedures were calculated as usual. The average values of measured efficiencies were consistent and are listed in Table 9. The same amounts of trichothecenes were exposed at room temperature for 7 days as before, extracted, cleaned up, and analyzed, and the recovery percentages are also listed in Table 9. These values seem to be lower than the recovery percentages of the unexposed samples. The loss of analyte due to adsorption during exposure, extraction, and cleanup processes is probably responsible for these low values. However, both values for each molecule were consistent, and with a correction factor, they could be easily analyzed with reasonable accuracy in real-world samples.

Table 9. Stability of Simple Trichothecenes

COMPOUND	AMOUNT ANALYZED (ng)	% RECOVERY AFTER 7 DAYS	% EXTRACTION & CLEAN-UP EFFICIENCY (DAYS) 0	7
3α-Hydroxyverrucarol (Scirpenetriol)	25-100	75±11	79±9	77±30
8β-Hydroxyverrucarol	"	73±9	58±9	29±0.0
8α-Hydroxyverrucarol	"	70±14	71±12	47*
16-Hydroxyverrucarol	"	83±13	46±13	42±18
Verrucarol	"	36±14	58±6	35±5
Deoxynivalenol	"	73±30	86±10	14±18
Fusarinon-X	"	75±20	70±22	-
Monoacetoxyscirpenol	"	46±12	66±6	29±13
Diacetoxyscirpenol	"	97±3	72±11	29±1
HT-2	"	54±7	64±16	20*
T-2	"	86±6	65±15	32*

*One value

Several spike (blank) experiments were conducted after establishing the optimum conditions for the extraction and cleanup of the trichothecenes and synthesis and analysis of their HFB esters.

Twelve commercial pollen samples, which were free from trichothecenes, were weighed (0.25 ± 0.01 gm) in 20-ml vials. The samples were then spiked with 10, 20, 40, 60, 80, or 100 ng of standards in duplicate, left exposed at room temperature for 24 hr, and then extracted with warm methanol (3 x 10 ml). The combined extracts were evaporated and cleaned up using silica-gel SEP-PAK cartridges. The residues were treated with internal standards, derivatized, and analyzed. A blank experiment was conducted along with the one with spiked samples. The results from the blank experiment confirmed that the commercial bee pollen was free from trichothecenes. The average recovery percentage of each trichothecene with respect to each internal standard was calculated from the results obtained with the 12 samples and is listed in Table 10. When HOVER was used as the internal standard, the values were much higher. The presence of derivatizable impurities in the pollen extract, despite cleanup, is probably responsible for reducing the efficiency in forming HOVER(HFB). In this case, DOVE was the better standard. However, a rigorous cleanup procedure is required for more accurate values when HOVER is used as the standard. The extraordinarily high values (over 200% in most instances) for T-2 in spiked bee pollen samples were due to interferences in the pollen. However, the identity of the interferents is presently unknown.

A dried papaya leaf was treated with 10 ml of warm methanol, kept immersed overnight, and decanted. A 50-µl aliquot was cleaned up, derivatized, analyzed, and found to be free of fusarium trichothecenes. Six different 50-µl aliquots of the extract were spiked with 10-100 ng of trichothecenes, cleaned up, and analyzed. These results are indicated in Table 10. All the trichothecenes, except DON, were recovered. Since the recoveries were consistent, with an introduced correction factor, the values could be applied for true samples. Both internal standards were considered adequate.

Similar experiments with spiked (blind) bee excrements were also conducted. The recovery percentages were calculated using the calibration data obtained from known spiked bee excrement samples. The measured recovery values of analytes in the blind samples were measured with at least 50% error and are probably due to insufficient cleanup of the samples.

Known amounts of DON, DAS, and T-2 were spiked on a blank (trichothecene-free) papaya leaf and left exposed in a bottle for 1 year. At the end of the year, the leaf was extracted with methanol. A 50-µl aliquot was cleaned up, derivatized, and analyzed. The same amount was also derivatized and analyzed without cleanup. The results from the experiment are indicated in Table 11. Since the spiking was done elsewhere and the exact

Table 10. Recovery Data of Spiked Samples

COMPOUND	Spike Range (ng)	PERCENT RECOVERIES			
		LEAF		POLLEN	
		DOVE	16-HOVER	DOVE	16-HOVER
3_α -Hydroxyverrucarol (Scirpenetriol)	20-100	46	33	43	62
8_β -Hydroxyverrucarol	"	51	38	86	153
8_α -Hydroxyverrucarol	"	43	12	126	203
Verrucarol	"	50	34	79	140
Deoxynivalenol	"	*	*	88	153
Fasarinon-X	"	54	42	99	164
Monoacetoxyscirpenol	"	73	67	88	160
Diacetoxyscirpenol	"	22	27	127	205
HT-2	"	63	48	47	72
T-2	"	29	41	**	**

*Less than 2%

**Extremely higher value due to interferences.

Table 11. Recovery of Simple Trichothecenes from Leaf Sample after Prolonged Exposure

COMPOUND	Amount Spiked* (µg)	Amount Detected (µg)	
		Unextracted	Extracted
Deoxynivalenol	10 ug	21.64	67.90
Diacetoxyscirpenol	5	1.24	3.80
T-2	5	2.36	8.18
HT-2	-	0.12	0.66

*Given value. Exact spike amount unknown.

amount of spiking was unknown, the only helpful information obtained from this experiment was that even after a prolonged exposure period, trichothecenes could be recovered from samples and identified using developed analytical procedures.

Several blind samples were received.* Some of them were hydrolysates of the samples, expected to contain macrocyclic trichothecenes, and the others were fusarium fermentation products. A few representative examples are shown in Table 12. The samples were cleaned up over silica gel cartridges. The eluants were evaporated and the residues were dissolved in 1 ml of methanol. A 1 to 2-µl aliquot of each of the samples was evaporated and treated with 100 ng of DOVE, derivatized as usual, and analyzed. The quantification values of the analytes corroborated the other results* obtained by either isolating the compounds from larger amounts of samples or by measuring the toxicity of the samples.*

The chromatograms of the sample labeled AHIVP93 are indicated in Figure 3. The relative retention times (DOVE) of the identified compounds in the sample agreed with those of the standards. Cultures of fusarium fungi (Bristol Laboratories, Strain 37410-90) grown on bee pollen* (Figure 4) contained T-2 and HT-2 toxins. Their presence was confirmed by recording their NICI (scan range, m/z 200-960) mass spectra (Figures 5 and 6). Another fermentation product* also contained low amounts of T-2 and HT-2 toxins. Quantification was not made for this sample since it was not requested.* A confirmation run as shown in Table 7 was made, and the presence of T-2 was established unequivocally (Figure 7). However, the presence of HT-2 could not confirmed by observing at least five characteristic ions (Figure 8). Hence, it was concluded that the presence of HT-2 toxin in this sample is suspected but not confirmed.

4. CONCLUSIONS

The experiments indicate that several naturally occuring trichothecenes and related compounds could be detected simultaneously and analyzed by the GC/NICI-MS method with excellent sensitivity and precision. Femtogram quantities of fusarium mycotoxins could be detected by the SIM mode. Low quantities (1-5 pg) of these analytes were sufficient to definitively identify and confirm trichothecene presence by this process. The investigated internal standards (DOVE and HOVER) were adequate for detecting and quantifying the toxins, even in low picogram quantities. Over a range of 6 orders of magnitude, the relative intensities of these trichothecenes varied linearly with their intensities' relative amounts with respect to the internal standard. The silica-gel SEP-PAK cleanup procedure used seemed adequate in certain cases. With added cleanup steps specific for various matrices, this method could become highly

*Personal communication with B. Jarvis of the University of Maryland (College Park, MD), 1983.

Table 12. Sample Analysis

Amount Sample	Compounds detected	Fraction derivatized	Amounts detected in Analyzed volume (pg)	Derivatized volume (ng)	Total sample (µg)
AHIVP93 (50 ml)	Scirpentriol	1/1000	21.0	10.5	10.5
	8α-Hydroxyverrucarol		312.0	156.0	156.0
	16-Hydroxyverrucarol		24.0	12.0	12.0
	Verrucarol		2418.0	1209.0	1209.0
MV3116 (50 ml)	Verrucarol	1/1000	18.0	9.0	9.0
Bee pollen	T-2 toxin	1/2000	2320.0	1160.0	2320.0
fermentation	HT-2 toxin		90.0	45.5	90.0
mixture (5 g)					
P8 (2.5 g)	8β-Hydroxyverrucarol	1/1000	42.0	21.0	21.0
	Verrucarol		14.0	7.0	7.0
	9,10-Epoxyverrucarol		34.0	17.0	17.0

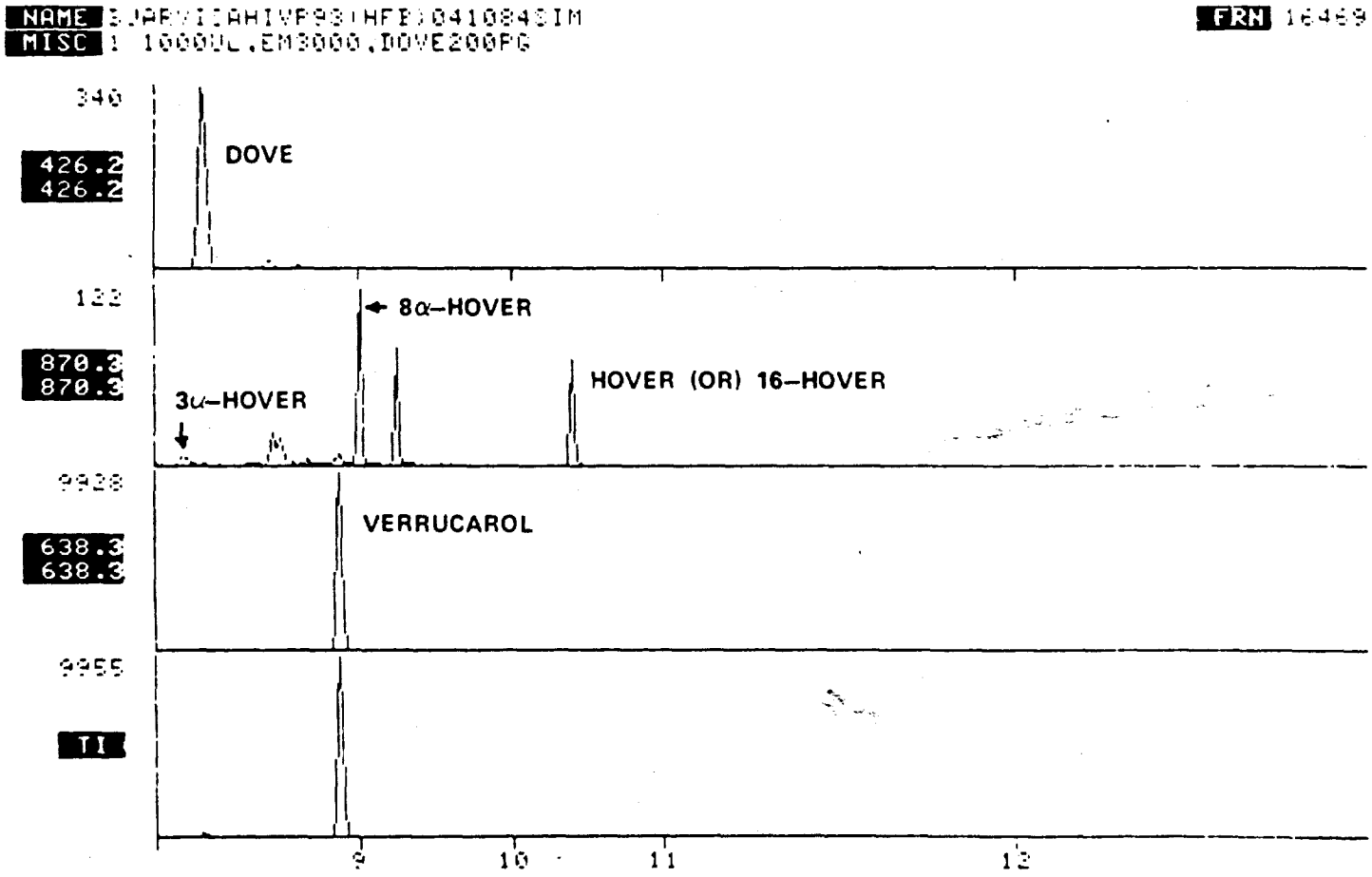


Figure 3. RIC of Fermentation Product AHIVP93 (HFB)

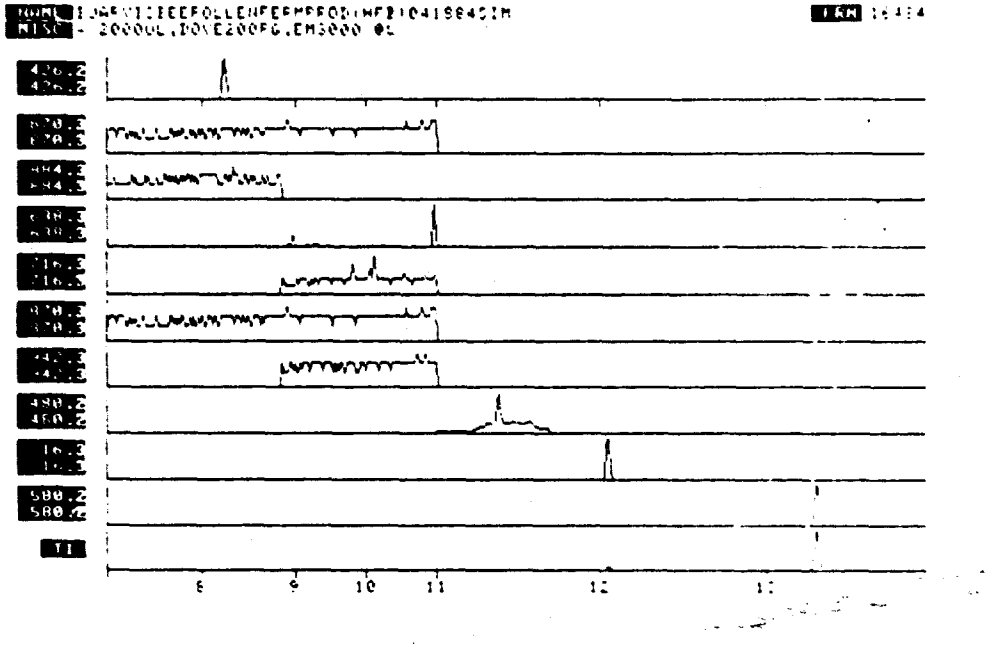


Figure 4. Screening of Bee Pollen Fermentation Product

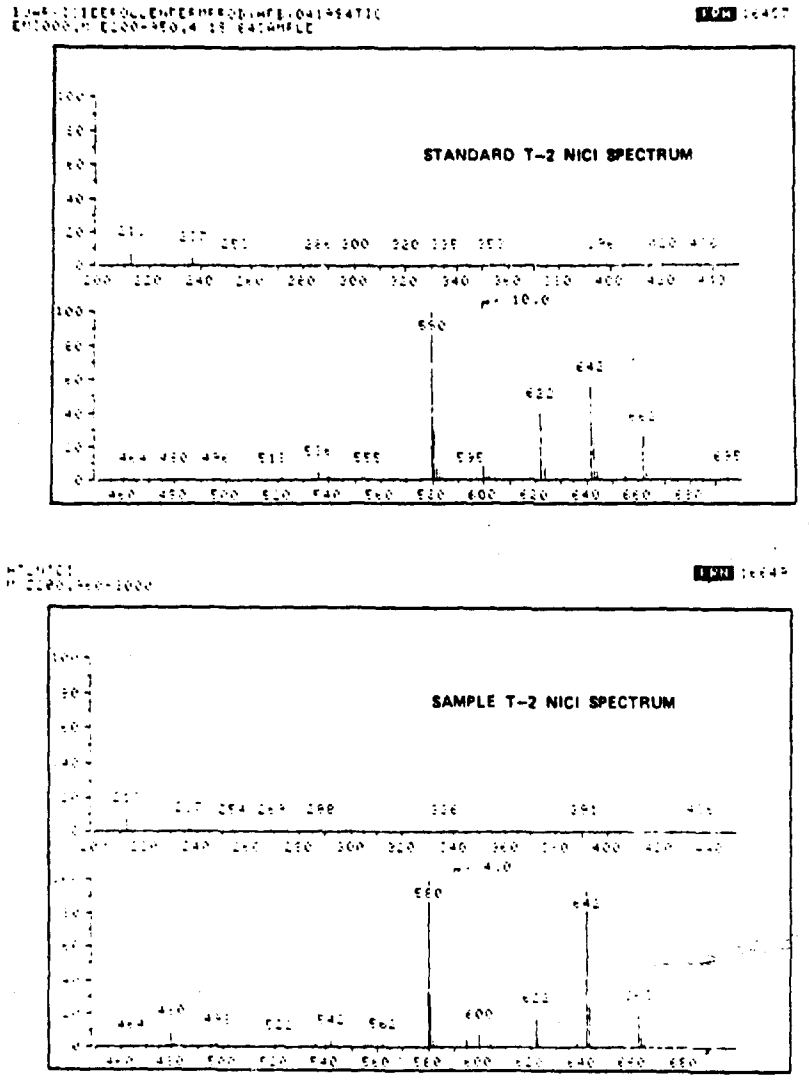


Figure 5. Confirmation of T-2 in Bee Pollen Fermentation Product by TIM

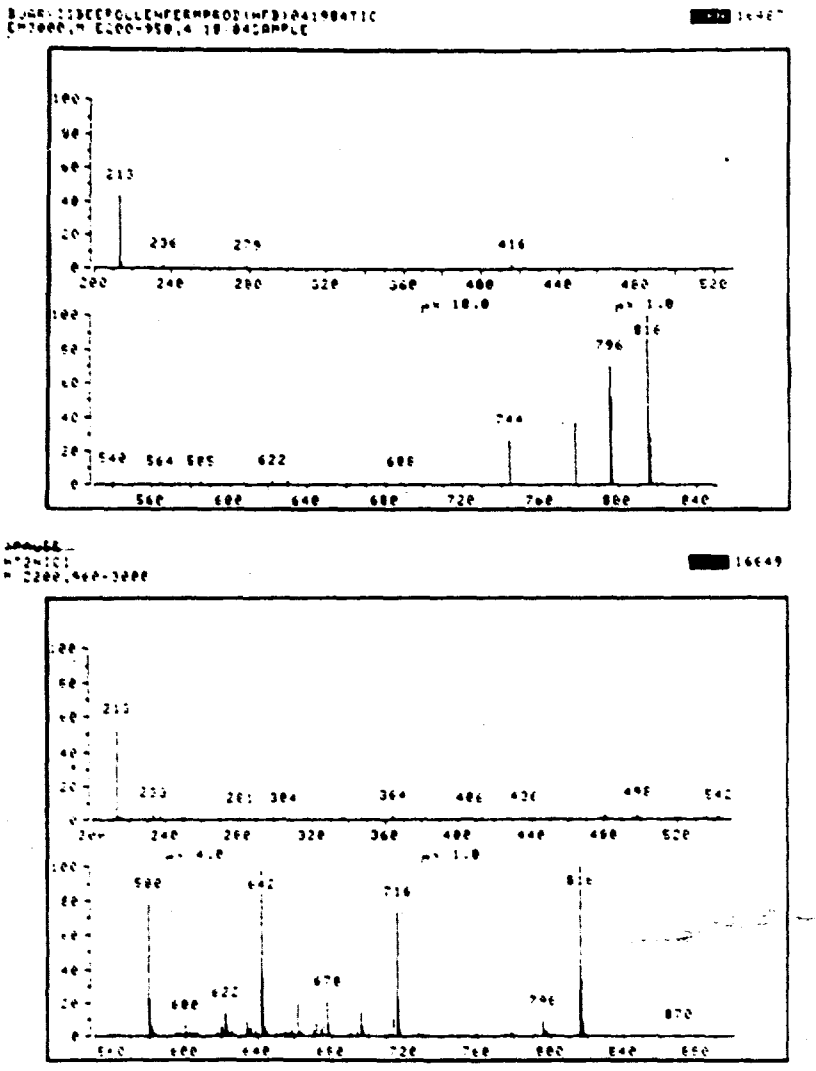


Figure 6. Confirmation of HT-2 in Bee Pollen Fermentation Product by TIM

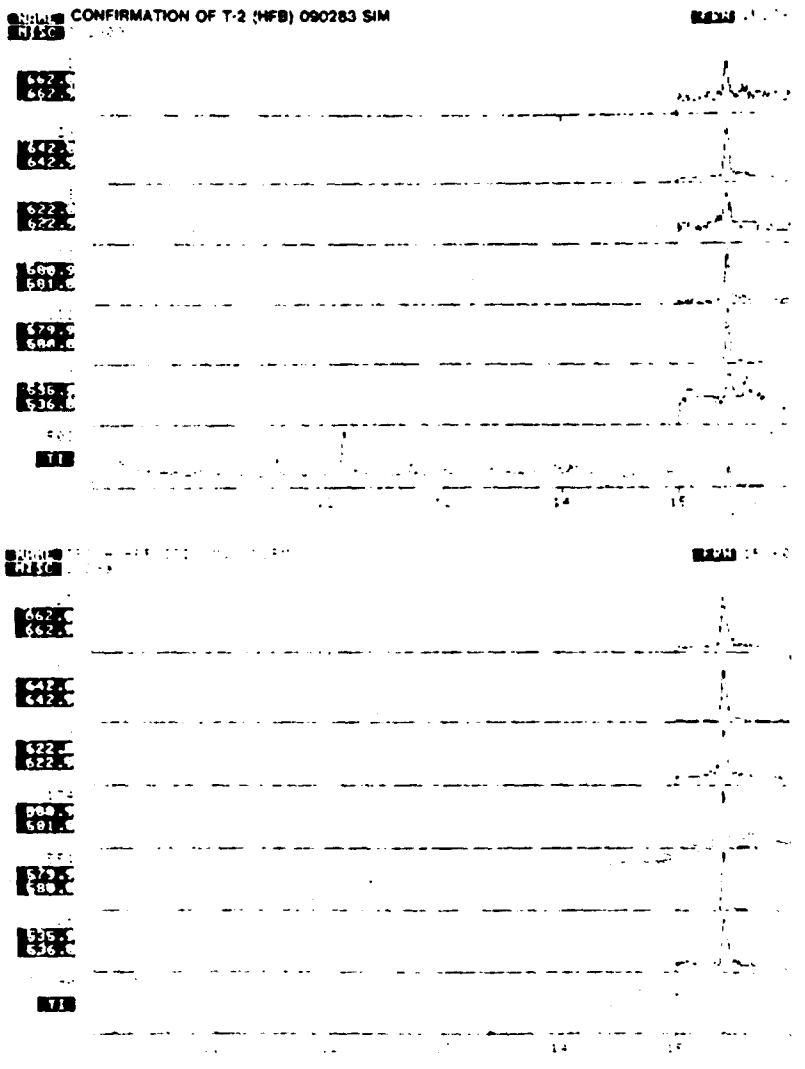


Figure 7. Confirmation of T-2 in Fermentation Broth by SIM

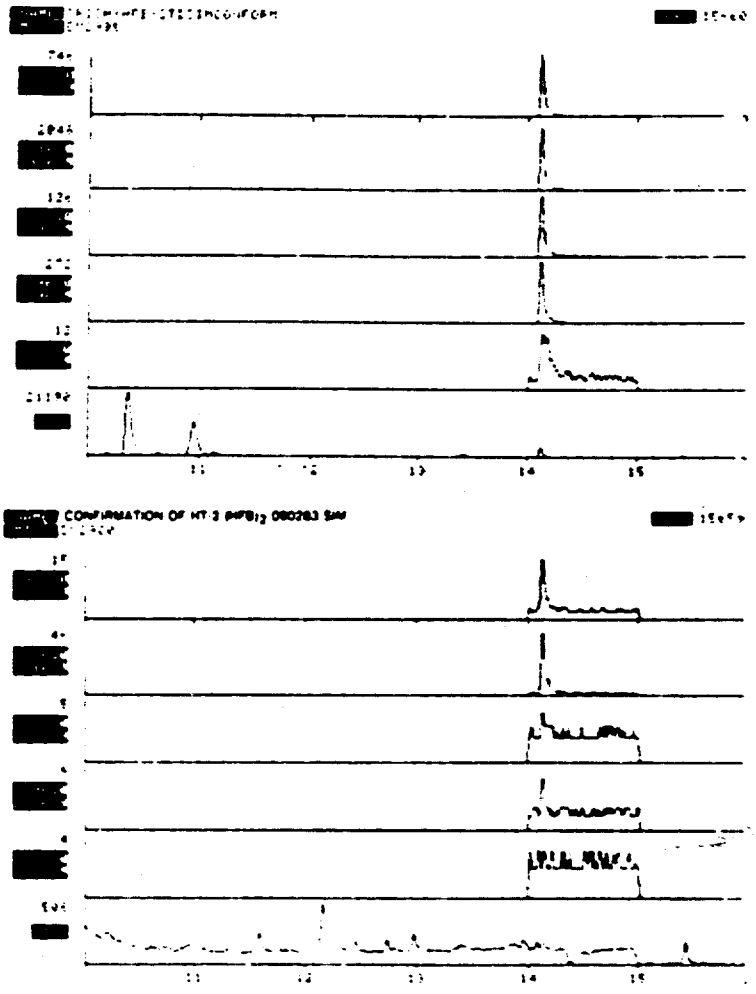


Figure 8. Confirmation of HT-2 in Fermentation Broth by SIM

beneficial for routinely analyzing real-world samples. The unknown samples are screened by SIM to detect 14 different simple trichothecenes simultaneously. When the analytes present are insufficient for confirmation by TIM, their presences are confirmed by the SIM mode, detecting at least five specific ions for each molecule. When combined with a cleanup procedure, this NICI method is a powerful tool for unambiguously identifying simple trichothecene mycotoxins and accurately quantifying real samples.

Blank

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